

Concept paper

Maximising the contribution of native-range studies towards the identification and prioritisation of weed biocontrol agents

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Abstract

Effective study in the native range to identify potential agents underpins all efforts in classical biological control of weeds. Good agents that demonstrate both a high degree of host specificity and the potential to be damaging are a very limited resource and must therefore be carefully studied and considered. The overseas component is often operationally difficult and expensive but can contribute considerably more than a list of herbivores attacking a particular target. While the principles underlying this foreign component have been understood for some time, recently developed technologies and methods can make very significant contributions to foreign studies. Molecular and genetic characterisations of both target weed and agent organism can be increasingly employed to more accurately define the identity and phylogeny of them. Climate matching and modelling software is now available and can be utilised to better select agents for particular regions of concern. Relational databases can store collection information for analysis and future enquiry while quantification of sampling effort, employment of statistical survey methods and analysis by techniques such as rarefaction curves contribute to efficient and effective searching. Obtaining good and timely identifications for discovered agent organisms is perhaps the most serious issue confronting the modern explorer. The diminishing numbers of specialist taxonomists employed at the major museums while international and national protocols demand higher standards of identity exacerbates the issue. Genetic barcoding may provide a very useful tool to overcome this problem. Native-range work also offers under-exploited opportunities for contributing towards predicting safety, abundance and efficacy of potential agents in their target environment.

Key words agent selection, efficacy of biological agents, foreign exploration.

INTRODUCTION

Foreign exploration in the native range of a weed is perhaps the most technically difficult and logistically time-consuming part of the biological control program. Yet, the entire outcome of a program depends on the suite of potential agents that is discovered. Further, the information gained during foreign exploration must contribute to the decisions as to which agents to select for full host-range testing and release. Prioritising agents with the greatest potential to control the target organism could minimise the numbers of species released in a program (Hoddle & Syrett 2002; Balciunas 2004). Limiting the number of species released may reduce risk to non-target species and

improve success (McEvoy & Coombs 1999; Strong & Pemberton 2001). In this paper we will discuss the information that can be gathered in native-range surveys that is useful in selecting agents for their environmental safety and potential efficacy. We have drawn heavily on our experiences in the tropics, but believe the challenges facing native-range surveying are universal.

Biological control programs have been conducted on the basis of very limited, relatively *ad hoc*, native-range surveying that look for abundant, damaging natural enemies (Goeden 1973; Klein 1999; Olckers 1999; Sparks 1999). However, where they have not resulted in complete success, sufficient data are generally not available to determine where future efforts should go. Conversely, other biological control programs have seen extensive, long-term native-range surveying efforts, which have yielded extensive checklists of natural enemies (Cordo & DeLoach 1987; Balciunas & Burrows

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1993; Harley *et al.* 1995; McClay *et al.* 1995; Palmer & Pullen 1995). However, they have sometimes provided little additional data with which to help prioritise agents, and it is often difficult to assess how comprehensive they were, and whether additional surveying would reveal additional natural enemies. There are therefore considerable opportunities to improve methods and approaches for native-range surveys.

This paper is structured around the twin aims of comprehensively listing the natural enemies on the target weed as expeditiously as possible, and obtaining data during the course of native-range surveys that will assist in their subsequent prioritisation. Comprehensive documentation of the native fauna will maximise the probability of locating an effective agent, help decisions regarding the optimal sequence of agent release (Denoth *et al.* 2002) and help in deciding when options for biological control have been exhausted for a particular target (Muller-Scharer *et al.* 1991). It is frequently not possible for surveying to be comprehensive, for a range of political, practical and financial considerations. However, most of the principles outlined in this paper should still form the basis of more restricted surveying efforts even if compromises need to be made.

THE IMPORTANCE OF THE FOREIGN COMPONENT

Successful biological control is predicated upon the use of effective organisms that can be safely introduced into new regions of the world. Traditionally safety has been the primary concern. However, there is now increasing pressure, through the scientific community and regulatory bodies, to also demonstrate that the potential agents are also likely to be highly efficacious. Although a target weed may have over 1000 phytophagous organisms associated with it in its native range (van Klinken & Campbell 2001), relatively few are likely to meet these criteria.

The number of organisms available as biocontrol agents for any given weed is a function of both the size and complexity of the native phytophagous fauna and the phylogenetic distance between the weed and valuable plants in the new region. While 41, 16 and 14 agents have been introduced for *Lantana camara*, *Opuntia stricta* and *Baccharis halimifolia*, respectively, where these plants have few close relatives in the introduced range, only 6 and 0 agents were available for *Acacia nilotica* (this has few close relatives) and *Senna obtusifolia*, respectively, when a very high degree of host specificity was required. Indeed some 522 agents have now been released against 260 weeds worldwide (Julien & Griffiths 1998), giving a crude estimate of approximately two agents per target weed.

Because only a very few organism are available for any given weed, those agents that can be utilised must be regarded as very valuable, but scarce resources and the major limiting factor for a biocontrol project. If for no other reason, this natural scarcity of suitable biocontrol agents justifies comprehensive searches throughout the native ranges for these potential agents.

Good faunistic studies of plants in the native habitat have considerable scientific value to disciplines other than biological control. For example, the insect community patterns and mechanisms elucidated by Strong *et al.* (1984) depended on a wealth of good faunistic studies. Many of these studies were undertaken in developed regions such as the UK and the USA. However, many of the studies undertaken for biological control are carried out in less developed areas of the world and therefore represent an opportunity to obtain valuable data to improve ecological knowledge. Improved surveying methodologies are, however, necessary if data are to be of wider value.

Prospective biocontrol organisms are usually first introduced into a quarantine facility where host-specificity testing is undertaken. Quarantine facilities have been greatly improved over the years and are regarded as giving a very high degree of safety against escape. This general upgrading of the facilities has allowed greater proportions of biocontrol risk assessment to be undertaken in them. It is now not uncommon for organisms to be introduced without any investigation in its native range. Although the quarantine facilities are adequate to support this practice, it remains a general principle that biosecurity is enhanced by the organism's being thoroughly studied in its country of origin before any importation into quarantine is made (Ferrar *et al.* 2004). In any case, native-range studies offer a wealth of opportunities for obtaining insights that will assist in agent prioritisation (and subsequent host-specificity testing, agent release and agent evaluation) that are not available under quarantine conditions.

WHERE TO SEARCH: DETERMINING THE NATIVE RANGE AND ORIGIN OF AN INVASIVE WEED POPULATION

Host-specific natural enemies are most likely to be found on the target weed within its native range, although biological control agents have been used from the target weed's introduced range (van Klinken & Julien 2003), and from congeners (Wapshere *et al.* 1989; van Klinken & Julien 2003) when host-specific requirements have been broad. It is also widely accepted that the centre of diversity for the genus and Pleistocene refugia should also be considered for exploration because natural enemies are likely to be most diverse and host-specific there (Schroeder & Goeden 1986; Wapshere *et al.* 1989; Muller-Scharer *et al.* 1991).

The native range 'is the area where a species occurs without having been introduced, deliberately or accidentally, by humans' (McClay *et al.* 2004). It can be difficult to distinguish between native and introduced ranges because distributional records are often scant and unresolved taxonomic issues are common. The process of determining the native range of a target weed begins through literature search and consultations with herbaria worldwide, but will often require additional taxonomic and/or molecular work. Once the native range of the target species is known, molecular methods can provide finer scale resolution as to the distribution and occurrence of genotypes.

New tools from molecular biology provide us with the ability to pinpoint the origin of an invasive species or populations (Chaboudez 1994; Radford *et al.* 2000). At a broader level, molecular characterisation can be used to avoid misidentifications of target species, especially in situations where the classical taxonomy of the group is not well known. Several programs have used random amplification of polymorphic DNA (RAPD) to look for matches or similarity (Ruiz *et al.* 2000). RAPDs amplify the entire genome of an organism including internal microflora, endosymbionts, etc. and therefore can be misleading phylogenetically and difficult to replicate (Molecular Ecology, policy on RAPDs). Sequencing parts of the genome using specific primers avoids these difficulties and the technology is readily available to scientists worldwide. Genes with high rates of mutation are best for detecting population-level differences, and can be used to match invasive populations with populations in their origin. Several genes should be sequenced from samples collected across the native range. The 'best' gene to use will be one that shows an adequate number of base pair changes to distinguish population-level differences. Common genes to sequence and their relative rates of evolution are shown in Table 1 (Cruzan 1998; Selkoe & Toonen 2006; PJ DeBarro, pers. comm. 2004).

Several genes, including D2, CO-1 and ITS-1, were used to determine the molecular phylogeny of *Lygodium microphyllum* but none showed sufficient genetic diversity to identify more than continental differences in populations (Goolsby *et al.* 2006). The two parts of the chloroplast genome, an intron between trnL and trnF genes, and the small ribosomal protein rps4 and trnS genes proved to be the most useful at distinguishing populations. Populations that were separated by biogeographical barriers had unique genotypes. This information proved to be critical to selection of the best-adapted genotype of the key biological control agent, the eriophyid mite, *Floracarus perrepae* Knihinicki & Boczek (Goolsby *et al.* 2004). The population of *L. microphyllum* from Cape York Queensland was an exact match with the invasive Florida population for all 1251 base pairs in the two chloroplast genes. The population of *F. perrepae* from this fern genotype performed best on the invasive Florida genotype of the fern, which supports the theory that intraspecific differences in genotype can be important (Kniskern & Rausher 2001).

Two DNA fragments, the trnL and the ITS-1, were used to identify eight of nine described subspecies of *A. nilotica* (L.)

Delile and to report a previously unknown genotype from Pakistan (Wardill *et al.* 2005). The study also indicated that the populations of this plant that are very serious weeds in Australia are mainly *A. nilotica* ssp. *indica* (Benth.) Brenan and that searches for biocontrol agents should therefore concentrate on the Indian subcontinent which is the native range for *A. nilotica* ssp. *indica*.

Confirmation of the identity of the introduced populations, at least to the species and preferably to population level, is imperative. There are several examples where matching native and introduced populations of the weed at subspecific levels has helped locate specific and/or damaging natural enemies at the species or genotype level including the eriophyid mite *Aceria chondrillae* (G. Can.) on skeleton weed (Cullen *et al.* 1982; Cullen & Moore 1983), the shoot bud gall on *Melaleuca quinquenervia* (Giblin-Davis *et al.* 2001) and the rust *Phragmidium violaceum* (Shultz) on blackberry (Evans *et al.* 2005) and conversely, where potential biocontrol agents collected from different subspecies to the target weed have been ineffective, for example, the acacia psyllid *Acizzia melanocephala* Burckhardt and Mifsud on *A. nilotica* (Palmer & Witt 2006). These close associations presumably reflect close host–enemy coevolution.

The more difficult situation arises when the invasive plant is a hybrid between two or more species such as is the case with salt cedar (*Tamarix* spp.) (Gaskin & Schaal 2003), lantana (Day *et al.* 2003) and mesquite (van Klinken & Campbell 2001). Differential performance by herbivores across hybrids and their component species is common, and specialist herbivores from the native range may not necessarily find the hybrid weed plant acceptable (Gaskin & Schaal 2003).

WHERE TO SAMPLE (LOCATION AND NUMBER OF SITES)

The fauna of the target weed will vary across the plant's native range in response to biogeographical barriers and variation in climate, habitat, land use and host density. Optimal sampling strategies are therefore required to maximise the probability of finding all the natural enemies and obtaining data that may assist agent prioritisation. Such strategies also need to account for practical and political considerations that might prevent access to parts of the native range. Geographical information

Table 1 Comparisons of commonly available genes and molecular techniques

D2 (16s ribosomal) – Nuclear gene, inexpensive, best for species-level differences in insects, sequences can be used for phylogenies
CO-1, CO-2 – Mitochondrial gene, best for species- and subspecies-level differences
ITS-1, ITS-2 – Nuclear ribosomal RNA gene complex, best for population-level differences, sometimes requires cloning which may increase cost
CpDNA, TrnL, RPS4 – Chloroplast genes that evolves rapidly, best for population-level differences
AFLP – Amplified fragment length polymorphisms, can detect variety-level differences
Microsatellites – Screen genome to find repeated sequences of genes expensive time-consuming to develop, good for population-level differences, that is, fruit flies, alleles presence or absence and allele frequencies which distinguish populations
EPIC – Exon Primed Intron Covering, like ITS-1, not as much variation as microsatellites, but good for population-level differences

systems, together with the growing availability of spatial data on a wide range of biophysical parameters, provide potentially useful tools for optimising sampling strategies.

The biogeography of a plant's distribution should be the first delineation of the search area. Plant populations that are separated by biogeographical barriers such as mountain ranges, deserts and oceans often have different and unique herbivores. For example, herbivore composition on *L. microphyllum* was strongly correlated with the biogeographical regions in which it occurred (Goolsby *et al.* 2003). Several of the lepidopterans were only collected from biogeographically isolated parts of the fern's range. *Austromusotima campozonale* was only collected from south-east Queensland and northern New South Wales. Conversely, the most widely distributed insect species, *Neomusotima conspurcatalis*, was collected from northern Australia and across Asia but not from south-east Queensland/New South Wales, or New Caledonia. The geographical and temporal isolation of New Caledonia may explain why the unique Pyraustinae species was found only on this island. The Malay Archipelago also showed high diversity of herbivore species. Several species including the stem-boring pyralid moth, *Ambia* sp., sawfly, *Neostromboceus albicomus*, and leaf-mining buprestid, *Endelus bakerianus* were found only in this area.

Plant species frequently occur over wider climatic conditions than do the herbivore and pathogen species that utilise them (Strong *et al.* 1984). Comprehensive surveys therefore require sampling across the whole climatic range of the host. Geographic features such as altitudinal gradients or rainshadows can provide opportunities for sampling diverse environments within relatively small areas (Carson & Okada 1983). Such data can also provide valuable insights into the ecology of the natural enemies (Zalucki and van Klinken, this issue).

Sometimes *a priori* decisions are made to limit searches to regions within the native range where the climate is comparable to the introduced range (Schroeder & Goeden 1986). Various eco-climatic matching approaches have been used (Wapshere 1974, 1983, 1993; Dennill & Gordon 1990), including the climate-matching function in CLIMEX (Sutherst *et al.* 2004) which allows site comparison based on rainfall, rainfall pattern, monthly average maximum/minimum temperatures, relative humidity and soil moisture (Kleinjan & Scott 1996; Adair & Scott 1997; Goolsby *et al.* 2004). However, agent selection based on climate matching can produce misleading results, depending on the species-specific climatic requirements of each insect and/or pathogen (van Klinken *et al.* 2003; van Klinken 2004). Also, the climate in the release environment is rarely exactly the same as that in the native range, and therefore it will always be difficult to predict agent performance based on climate matching alone. It is therefore advisable to conduct surveys throughout the native range, including regions that are climatically different from the introduced range.

All habitat types in which the target plant grows should be surveyed. Maps which distinguish habitats are generally available for most parts of the world and combine many eco-

logical factors such as soil type, climatic conditions, topography and vegetation structure and composition (e.g. http://audit.ea.gov.au/anra/atlas_home.cfm). In the *L. microphyllum* biocontrol program published vegetation maps were used to identify unique habitats within biogeographical areas for exploration. In Australia, the target plant was located in four different habitat types, wet and dry sclerophyll, eucalypt woodland and rainforest. Perhaps the most interesting habitat proved to be the rainforest of Cape York. This area of Australia is known to have strong faunistic affinities with New Guinea; therefore, it represented both a new habitat and biogeographical region within Australia. This was significant because the Cape York population of *L. microphyllum* was different from the rest of Australia and a genetic match for the invasive Florida population.

The number of locations and sites that need to be surveyed will depend in part on the heterogeneity of the fauna of the target plant. Some initial replication of sites will provide an indication of local variation in the fauna, and whether additional sites are likely to yield further species (Muller-Scharer *et al.* 1991).

WHAT SITE DATA TO RECORD?

Considerable data can be recorded about each collecting site. Good data will provide a better understanding of the environmental conditions in which the weed can grow within the native range, including the identification of possible factors regulating its abundance (Lonsdale & Segura 1987; Paynter *et al.* 2003). It can also help in identifying environmental preferences, or constraints to distributions, of potential agents. For example, soil type, temperatures and inundation can be important for species that pupate in the soil (Palmer & Haseler 1992). Finally, it can be used to assess sampling effort across environmental gradients, and help identify priority gaps for additional surveying.

Basic site description data, such as name, locality, latitude/longitude, topography, soil type, inundation frequency and duration, and vegetation type are typically recorded, as are accession numbers for herbarium and DNA plant samples and photographic documentation. Other ecological data can also be collected depending on the questions of interest. They include weed parameters (height, reproductive state, density, age structure), the presence of close relatives and land use history. A range of protocols and tools are available that can help quantify many of these variables (McDonald *et al.* 1998). Site data together with survey data and results should be entered into a relational database such as Biota® (Colwell 2004) or that of Palmer (1995). Relational databases not only allow the quick and easy future recall of information, but also allow analysis and comparison of data. For example McClay *et al.* (1995) were able to calculate pairwise indices of faunal similarity for *Parthenium hysterophorus* and 11 other taxa while Palmer and Pullen (1995) were able to analyse the contributions of four separate searching efforts for agents for lantana.

QUANTITATIVE AND SYSTEMATIC METHODS FOR NATIVE-RANGE SURVEYS

Quantitative and systematic survey methods need to be tailored for each target weed to minimise potential biases in the guilds being surveyed and provide data to determine when sufficient samples have been taken by methods such as rarefaction curves (Muller-Scharer *et al.* 1991).

Systematic sampling can be particularly difficult on large and structurally complex plants such as trees and shrubs that require considerable effort to survey all plant parts. Different life stages may also need to be explicitly targeted, including the seed bank and seedlings, as they can yield a different suite of natural enemies. However, sometimes *a priori* decisions are made not to specifically target particular plant parts, feeding guilds or taxa, such as roots and seedling feeders of mesquite (Cordo & DeLoach 1987) or flower feeders on prickly acacia (Marohasy 1995; Kriticos *et al.* 1999). These decisions may be based on practical considerations such as available expertise, the *a priori* identification of particular guilds that were considered unlikely to be effective (Harley *et al.* 1995; Kriticos *et al.* 1999) or because only selected guilds would be approved for release (Impson *et al.* 1999).

Several methods have been employed for sampling natural enemies, including visual searches for organisms or signs of damage, sweeping, beating, and destructive sampling for subsequent breeding, extraction or examination in the laboratory. Specific methods or expertise are required for different taxa of insects, mites and pathogens (Andres *et al.* 1976). However, some methods, such as sweeping, can be less discriminatory, and therefore risk collecting diverse species for which the target is not a host, including predators, pollinators and vagrants. Molecular techniques increase the value of collecting immature stages, such as root and stem borers, that are likely to be difficult to culture and difficult to identify as larvae or pupae. Any evidence of damage on the target plant, including defoliation, gall formation, flower and fruit damage or dead tissue, can be useful for determining the phenology of herbivore species, at least when damage can be associated with particular species.

Quantification of sampling effort on each visit is desirable to provide data on the density of herbivores or pathogens that can be used in developing models to predict distribution and abundance in the native range (Scott 1992; Zalucki *et al.*, this issue). Various methods have been or can be used, including plant-based methods, time-based methods (Goolsby *et al.* 2003) and fixed or random transects. Plant-based methods include searching a fixed number of plants per visit (Marshall *et al.* 1981; Schroeder 1985; Schroeder & Goeden 1986), and collecting a fixed number of pods from each developmental stage (van Klinken 2004). Sometimes within-site stratification of sampling, such as across different plant densities, inundation levels or age structures, may be useful.

The time of day in which field surveys are conducted can potentially affect the results, especially for dispersive stages. Although night time would reveal a different suite of species,

it would be fair to say that most surveys give insufficient attention to that period for various and understandable reasons (employment of helpers, inaccessibility and safety issues). Infra-red night vision glasses may overcome some of these problems. In cool regions, insects may only be active during warmer parts of the day.

Field sites should ideally be visited several times during the year over several years to collect the full diversity of herbivores, as species will almost certainly have different phenologies, and abundances can vary from year to year, especially with so-called outbreak species. The timing should reflect the obvious seasonal differences and phenological phases of the plant, but also take into account weather events for opportunistic surveys. Collections made shortly after periods of high rainfall are often very productive.

The benefits of quantitative surveying methods are illustrated by the native-range surveying of *L. microphyllum* (Goolsby *et al.* 2003). All the geographically unique areas were visited several times and during wet and dry seasons. Several sites across the native distribution were systematically surveyed every month to test whether rare species were going undetected, and also to provide data on seasonal phenology of the herbivores and their natural enemies (predators, parasitoids and pathogens). A total of 513 field collections were made across Australia and South-East Asia, and results entered into a Biota database (Colwell 2004) (Sinauer Associates, Inc, Sunderland, MA, USA). Analysis showed that many of the herbivores were only rarely observed, including the stem borer, *Ambia* sp., that was collected only three times. In nearly half of the collections (248), no herbivore or only the ubiquitous *F. perrepae* was collected. Approximately 500 h was spent at collection sites over a 3-year period to collect the 20 herbivore species reported in Goolsby *et al.* (2003). (In comparison, in a similar program for the paperbark tree, *M. quinquenervia*, more than 450 herbivores were collected during a similar period of time (Balciunas & Burrows 1993).) The data in Figure 1 indicate strongly that all above-ground herbivores were collected, including rare herbivores with restricted distributions, as no additional herbivore species were found in the additional 300 h of surveys from 2002 to 2004.

Herbivores that at least occasionally are seen to be damaging in the native range (sometimes as a result of an 'outbreak') are considered to be good candidates as biological control agents (Wapshere 1985). This is because if they are able to reach damaging levels in the presence of their own natural enemies, they will likely do the same in the introduced range where the local natural enemies will be less adapted. From our experience, with *L. microphyllum*, the only herbivore that consistently occurred at high population levels was the mite, *F. perrepae*. Outbreaks were also reported across its range, including Australia and Asia. Insect outbreaks were extremely rare. We observed only one outbreak in 3 years for the moth, *A. camptozonale*. This moth consistently occurred at very low densities in south-eastern Queensland. Therefore, we prioritised the mite because it was frequent in outbreak populations causing considerable damage to the target fern.

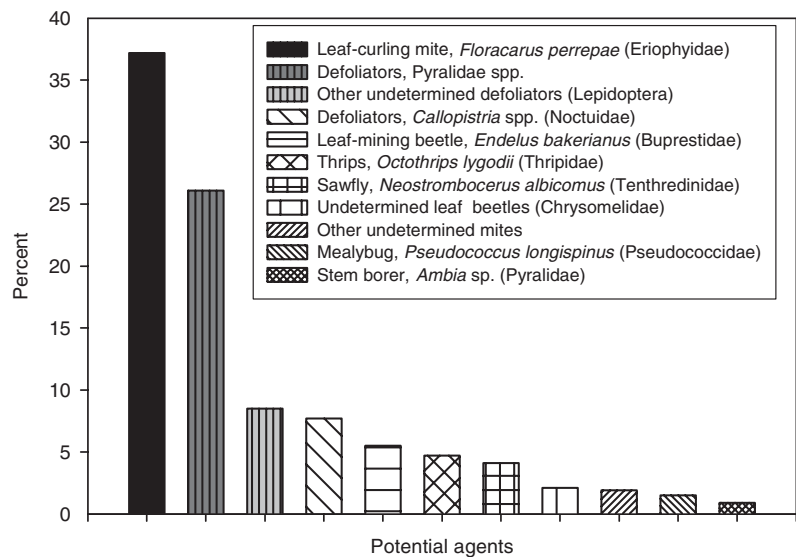


Fig. 1. Relative abundance of *Lygodium microphyllum* herbivores in field collections ($n = 513$), based on total number of herbivores collected from 1999 to 2002.

IDENTIFYING THE NATURAL ENEMIES: DEALING WITH THE TAXONOMIC IMPEDIMENT

Accurate and timely identification of herbivores and pathogens presents one of the biggest obstacles to native-range surveying and the subsequent prioritisation of agents. Fauna is typically poorly known, especially in less studied regions with high levels of biodiversity, such as the Neotropics. Taxonomic expertise is generally required across diverse taxa, and waits of several years for identifications are now common (Heard & Pettit 2005). Even so, reliable identification beyond the genus level, or higher, is often not possible without a full revision of the group in question, sibling species can remain undetected and misidentifications can still occur. A result is that a high proportion of the recorded fauna remains unresolved at the species level and many true species probably remain undetected. Perhaps more seriously, it means that other data on these unidentified species from the published literature cannot be utilised because of uncertainty as to identity of the species collected.

Molecular methods, used in tandem with classical taxonomy, potentially provides a way to rapidly assign species with unique markers with which to associate biological information. Taxonomists can relatively rapidly assign material to morpho-taxonomic units and classify them to the family level, or even lower for some groups. Molecular techniques of appropriate resolution to differentiate material at the species level (e.g. see Table 1) would then help determine whether the morpho-taxonomic unit represents more than one species, and to assign a genetic barcode to each species. Sequence data can be posted on GenBank (<http://www.ncbi.nlm.nih.gov/>), which serves as a molecular voucher library.

The use of genetic barcoding has attracted considerable controversy in the literature (Herbert *et al.* 2003; Sperling 2004), partly because of the difficulty of determining what

level of genetic divergence represents a new species, and because divergence levels are expected to be taxon-specific. At least some of these problems can probably be overcome through the use of more than one molecular technique, and more detailed morphological examination of the material, as required. For high-priority species, several genes with rapid rates of evolution should be sequenced so that the genus-, species- and population-level differences can be assessed. This information can be used to develop the phylogeny of the species or populations.

Molecular tools have already been found to be highly effective in resolving taxonomic issues of particular taxa. The leaf defoliating moth, *Callopietria* sp., occurred over most of the native distribution of *L. microphyllum*. Molecular sequencing revealed that it was a complex of three separate species. Conversely, *N. conspurcatalis* occurred over a similar distribution but was found to be the same genotype in all areas where it was collected. Similarly, molecular tools have been used to match immature stages with adults that have been collected from sweeping or hand searches. This has previously been a common constraint to identifying stem borers and other difficult-to-rear species. This tool was used to identify the lygodium stem-boring moth *Ambia* sp. which was only commonly collected in the field as an immature and matched with the adult moth by sequencing only the leg of the single specimen.

Where used, molecular methods have been relatively cost-effective, as they rely on commonly practised molecular techniques. Costs are likely to become cheaper as techniques become increasingly automated. Multiple specimens from each location should be characterised to ensure consistency in the results.

Software programs, such as LucID (ISBN 0643 06415 X), are now available to assist in organising morphological, genetic and biological data. They are likely to be particularly valuable where faunas are diverse and surveying is being conducted across a wide geographical area.

NATIVE-RANGE STUDIES OF THE HERBIVORES AND TARGET WEED

A wide range of additional data can be collected from the native range, which can further help prioritise agents. Some of this work is contingent on the availability of local field stations and/or collaborators while some can be done through the course of general surveying work. We discuss the types of data that can be collected which will help predict host specificity, likely distribution and abundance, and possible impacts of potential agents within the release environment. Predicting population dynamics and impacts of specific agents receives further attention elsewhere in this issue (Zalucki *et al.*, this issue; Raghu and Dhileepan, this issue).

Host specificity

Useful information on host specificity can be obtained from the native range, although caution is required to ensure that records on other species do not represent a complex of sibling species (Robinson 1985; McClay *et al.* 1995) or that the putative host has not been misidentified (Palmer & Diatloff 1987). Also, host observations in the native range that the insect has only a limited host range do not guarantee host specificity in the release environment (van Klinken 2000), but they can be a good guide.

Valuable field host-specificity data can be obtained through surveying close relatives growing sympatrically with the target at the survey sites (Witt 2004). Alternatively, closely related species and key non-target species from the introduced range can be planted in randomised plot designs, which allows for more stringent statistical analysis of data. Several manipulative field plot trial designs have been used and have been summarised (Briese 1999; Briese *et al.* 2002). Herbivores in these studies are also free to disperse, aggregate and reveal other behavioural attributes that influence their host selection, preference and utilisation. In the *L. microphyllum* program the garden plot studies were part of a continuum of host-range studies from micro-sized sporeling ferns, to potted greenhouse plants, to large, field-grown plants. The field plot study supported the conclusion that *F. perrepae* is a specialist on *L. microphyllum*. This approach eliminated the need to import all the mite genotypes into quarantine, which may have added an additional biosecurity risk.

In some cases native-range field surveys can also be combined with laboratory studies to obtain detailed information on specific natural enemies. For example, a mobile host-specificity testing method was developed for eriophyid mites of *L. microphyllum* and used to test whether different genotypes of the mite *F. perrepae* differed in their host specificity (Goolsby *et al.* 2004; Goolsby *et al.* 2006). This method proved useful in screening mite genotypes for their ability to induce leaf curls and reproduce on the invasive Florida genotype of *L. microphyllum*. Sporelings were transported to China, India, New Caledonia and Thailand to test the local genotypes of *F. perrepae*. Field-collected mites from these locations were transferred to the sporelings and within 2–3

weeks it could be determined whether the mites were able to feed and reproduce on the target genotype of *L. microphyllum*.

Phenology

Repeat surveys at select sites in the native range can provide phenological data on key herbivores which, in turn, can help identify factors underlying seasonal dynamics of potential agents that may influence their success in release environment. Phenological data are more useful if quantitative (Scott 1992) than if just presence/absence (Zalucki *et al.*, this issue).

Surveys over a 2-year period on *L. microphyllum* in south-eastern Queensland measured the density of leaf roll galls, numbers of *F. perrepae*, predators and pathogens in the leaf rolls and correlated their phenology with several climatic variables. The field studies showed that the mite was active year round, with populations peaking when temperatures were cool and soil moisture levels were highest. Throughout the 2-year field study, *F. perrepae* caused consistent damage to *L. microphyllum* at all the field sites. Similar data from India and New Caledonia suggested that populations of the mite were depressed by heavy rainfall and that the incidence of leaf rolls fell when the mean temperature rose above 27°C, and ceased above 35°C. However, the weather parameters in Homestead, Florida are within the range of those evaluated in the native range, and therefore it was concluded that climate would not prevent the establishment of *F. perrepae*, and that it would still be effective even with relatively high levels of predation or disease.

Natural enemies

A common assertion in biological control is that potential agents will be more abundant in their introduced range because they will be released from their natural enemies, especially if natural enemies are specialists. Indeed, Lawton (1985) considered that a principal criterion for selecting an agent is that it should differ sufficiently from local herbivores, both taxonomically and in method of attack on its plant host, that it would remain 'enemy free' once introduced (Wapshere *et al.* 1989). Enemy-free space is not, however, essential for an agent to be effective. For example, agents can be very abundant even with relatively diverse parasitoid faunas (van Klinken & Burwell 2005), and high levels of predation and parasitism (Bess & Haramoto 1972). Nonetheless, an understanding of susceptibility to natural enemies is of considerable value when prioritising agents (Zalucki *et al.*, this issue).

For both the *M. quinquenervia* and *L. microphyllum* bio-control programs the natural enemies of candidate biological control agents were studied. The melaleuca gall fly, *Fergusonina turneri*, appeared to have many of the attributes of an effective gall maker, that is, high functional response, heavily lignified gall, and the ability to block reproduction and bud growth of the target weed (Goolsby *et al.* 2000). However, the gall generally occurred at low density in the native range, presumably due to high levels of predation by specialist natural enemies. Goolsby *et al.* (2001) determined that 7 of the 11 gall inhabiting Hymenoptera were primary parasitoids, and

one had a specialised predatoid biology which allowed it to chew through plant tissue to consume multiple *F. turneri* immatures. There was therefore excellent potential for the gall fly to reach higher, damaging population levels in the introduced range where it would be free from its suite of specialist natural enemies.

Similarly, the mite, *F. perrepae*, appeared to cause significant damage to *L. microphyllum* in the native range despite its association with several mite predators and pathogens. Ozman and Goolsby (2005) documented the impact of these natural enemies of *F. perrepae* in south-eastern Queensland. Although the natural enemies had significant impact on the population levels of the mite, significant plant damage was sustained over the 2-year study. It was concluded that even if the impact of natural enemies in Florida were similar to that experienced in the native range, the mite could still be an effective biological control agent and if the impact of predators and pathogens in Florida were less than that experienced in the native range the mite could reach higher population levels.

Herbivore impact

Limited impact data can be obtained, or at least inferred, directly from surveys. However, the degree of damage, and therefore impact, is not necessarily a good predictor of what will happen in the introduced range as that will depend on the abundance reached there (Wapshere *et al.* 1989), and whether the plant will respond in the same way in the release environment. However, valuable data can be obtained through more intensive studies within the native range, including comparative plant ecological studies and efficacy studies (e.g. insect exclusion trials and releases of agents into test plots).

Comparative studies between weed populations in the introduced and native range can provide insights into factors that might be limiting populations within the native range (Lonsdale & Segura 1987; Paynter *et al.* 2003). These types of studies were conducted on *M. quinquenervia* over a 6-year period in both the native and introduced range. Investigations focused on the regeneration potential of *M. quinquenervia*: including biomass allocation, stand biomass, flower phenology, seed production, seed set and seed viability, rate of seed rain, seedling survival and stand demographics in Australia for comparison with plant characteristics in Florida (Rayachhetry *et al.* 1998). Populations in Australia were much less vigorous than those in Florida, with much higher levels of flower and bud abortion, fewer seeds and less viable seeds. Also, seedling survival was substantially lower, apparently because of attack by the cecid gall maker, *Lophodiplosis trifida*. This information provided a rationale to prioritise agents such as the gallfly, *F. turneri*, that attacked the developing reproductive structures of *M. quinquenervia* and the cecid gall-maker, *L. trifida*, which targets seedling plants.

Chemical exclusion trials are more resource-intensive, and can help determine the impact of herbivores at the densities at which they occur within the native range (Walloff & Richards 1977). This may be useful for potential agents that are relatively abundant in the native range. Such a study was per-

formed with *L. microphyllum* in its native range to assess the impact of the dominant herbivore, *F. perrepae*, on biomass production. In a 2-year garden plot study using chemical exclusion methods, *F. perrepae* caused a significant reduction in biomass of above-ground stems and leaves and below-ground roots and rhizomes. Populations of native predator mites were low throughout the study; however, the mite pathogen *Hirsutella thompsonii* Fisher was common in the second year of the study, but neither reduced the impact of *F. perrepae*. Based on its potential to cause significant damage to *L. microphyllum* under field conditions in the native range, this agent was prioritised for release in Florida.

Experimentally manipulating densities of potential agents on their hosts in the native range can be used to evaluate the impact of selected agents that may be resource limited. Some agents can be resource limited in the native range, because their host plant may be relatively uncommon. Native trap gardens of the target plant at high density may be used to overcome the hurdle of resource limitation and quantify what might happen if the agent reached high densities in the introduced range. Briese (1996) evaluated the impact of a resource-limited stem-boring weevil, *Lixus cardui*, on seed production of the *Onopordum* thistles using garden plots. Sheppard (2003) further discusses the use of per capita impact methods to prioritise agents for agent selection.

CONCLUSIONS

The overseas component of a biological control project continues to be the cornerstone on which all other investigations ultimately depend. Expenditure of effort on this overseas component maximises the chances of finding potential agents, adds to our knowledge of flora and fauna in the overseas country, enables sensible prioritisation of potential agents, improves the efficiency of research in the target country, improves the probability of eventual establishment of successful agents and decreases the risk of introducing unwanted organisms. It is therefore no coincidence that most of the successful biological control projects have had a significant overseas component while those with only an *ad hoc* approach to the overseas component have not consistently produced effective results.

Ideally this component should be undertaken from permanently staffed overseas field stations so that surveys can be undertaken throughout the year and be supported by the range of studies and techniques, described in this paper, that are most appropriately undertaken in the native range of the organisms. It is very clear that much more than simply looking for and listing potential agents can be achieved by working in the native range. This paper indicates that good science, using a range of modern techniques, can be practised to produce results worthy of international publication.

The overseas component of biological control has never been without its difficulties and the present is no different. When Albert Koebele arrived in Mexico in 1902 to search for lantana insects he encountered cholera, very difficult shipping

arrangements for his insects, no taxonomic support, and was almost recalled after a few weeks because he had not produced results (Perkins & Swezey 1924). Today there are issues of funding and having staff work in difficult overseas situations. The diminishing numbers of taxonomists worldwide, and the increasing difficulty of getting organisms correctly and timely named remain very serious issues.

Native-range surveys can be designed to provide a comprehensive list of potential agents, as well as additional data to prioritise them for safety and efficacy. Sometimes comprehensive surveys are not undertaken for practical, political and financial considerations or when the importance of specific guilds or climatic conditions has been identified *a priori*. Any such gaps and assumptions need to be fully documented for future workers. Optimising survey methodologies also offers considerable efficiency gains, especially in determining where, when and for how long surveys need to continue. Developments in molecular systematics and taxonomy, and the increasing availability of spatial biophysical data and off-the-shelf databases designed to handle bioinformatic data, present a range of as yet rarely exploited opportunities for improving the value of native-range surveying activities.

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